Synthesis and characterization of some water soluble Zn(ii) complexes with (E)-N-(pyridin-2-ylmethylene)arylamines that regulate tumour cell death by interacting with DNA

Basu Baul, Tushar S ; Kundu, Sajal ; Linden, Anthony ; Raviprakash, Nune ; Manna, Sunil K ; Guedes da Silva, M Fátima C

Abstract: The synthesis and spectroscopic properties of nine water soluble zinc(II) complexes of (E)-N-(pyridin- 2-ylmethylene)arylamines (Ln) with the general formula [Zn(X)2(Ln)] (X = Cl−, Br−, I−; (1–8)) and [Zn(-N3)- (N3)(L3)]2 (9) are reported. The complexes were characterized by elemental analysis and their spectroscopic properties were studied using UV-Visible, fluorescence, IR and 1H NMR spectroscopies. The solid state structures of zinc(II) complexes 2–4 and 6–9 were established by single crystal X-ray crystallography. The majority of the structures are mononuclear with tetra-coordinate zinc centres (2–4, 6 and 7) except where L carries an additional donor atom capable of coordinating zinc (8), in which case the zinc atom has a distorted square pyramidal geometry. The centrosymmetric molecule of [Zn(-N3)(N3)(L3)]2 (9) is binuclear with the zinc atoms in a trigonal bipyramidal coordination environment. In general, the dichlorozinc derivatives 1, 3–5 and 8 exhibited moderately elevated in vitro cytotoxic potency towards the human epithelial cervical carcinoma (HeLa) cell line, with 4 as the best performer (IC50 value of 18 M). Apoptosis-inducing activity, assessed by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay, showed that the zinc complexes interacted with DNA and thereby interfered the DNA binding of several transcription factors to its promoter sites, thus inhibiting gene transcription required for the biological activity of cells.

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Synthesis and characterization of some water soluble Zn(II) complexes with (*E*)-*N*-(pyridin-2-ylmethylene)arylamine that regulate tumour cell death by interacting with DNA†

Tushar S. Basu Baul,[a,*] Sajal Kundu,[a] Anthony Linden,[b] Nune Raviprakash,[c] Sunil K. Manna[c] and M. Fátima C. Guedes da Silva[d,e]

Abstract

Synthesis and spectroscopic properties of nine water soluble zinc(II) complexes of (*E*)-*N*-(pyridin-2-ylmethylene)arylamine (L^n) with general formula $[Zn(X)_2(L^n)]$ (X = Cl^-, Br^-, I^-; (**1** - **8**)) and $[Zn(\mu\text{-}N_3)(N_3)(L^3)]_2$ (**9**)) are reported. The complexes were characterized by elemental analysis and their spectroscopic properties were studied using UV-Visible, fluorescence, IR and ^1H NMR spectroscopies. The solid state structures of zinc(II) complexes **2**-**4** and **6**-**9** were established by single crystal X-ray crystallography. The majority of the structures are mononuclear with tetra-coordinate zinc centres (**2**-**4**, **6** and **7**), except when L carries an additional donor atom capable of coordinating zinc (**8**), in which case the zinc atom has a distorted square pyramidal geometry. The centrosymmetric molecule of $[Zn(\mu\text{-}N_3)(N_3)(L^3)]_2$ (**9**) is binuclear with the zinc atoms in a trigonal bipyramidal coordination environment. In general, the dichlorozinc derivatives **1**, **3**-**5** and **8**, exhibited moderately elevated *in vitro* cytotoxic potency towards the human epithelial cervical carcinoma (HeLa) cell lines, with **4** as the best performer (IC_{50} value of 18 μM). Apoptosis-inducing activities, assessed by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay, showed that the zinc complexes facilitated the interaction with DNA interfering with the binding of several

transcription factors to its promoter sites, thus inhibiting gene transcription required for the biological activity of cells.

[a]*Department of Chemistry, North-Eastern Hill University, NEHU Permanent Campus, Umshing, Shillong 793 022, India; E-mail: basubaul@nehu.ac.in, basubaulchem@gmail.com; Fax: +91-3642721000; Tel: +91-3642722626*

[b]*Institute of Organic Chemistry, University of Zurich, Winterthurerstrasse 190, CH-8057 Zurich, Switzerland*

[c]*Laboratory of Immunology, Centre for DNA Fingerprinting and Diagnostics, Gruhakalpa, Nampally, Hyderabad 500001, India*

[d]*Centro de Química Estrutural, Complexo I, Instituto Superior Técnico, University of Lisbon, Av. Rovisco Pais, 1049-001 Lisboa, Portugal*

[e]*Universidade Lusófona de Humanidades e Tecnologias, ULHT Lisbon, Av. do Campo Grande, 376, 1749-024 Lisbon, Portugal*

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Introduction

Metal complexes of α-diimine Schiff bases have found increasing applicability as catalyst precursors for olefin polymerization. In this perspective, the pioneering work of Brookhart, Bennett and Gibson have shown that tridentate 2,6-bis(arylimino)pyridyl metal (Fe or Co) dihalides, upon activation with methylaluminoxane (MAO), led to effective catalysts for the conversion of ethylene to high-density polyethylene or to α-olifins.[1] Apart from possible structural disparity of the metal complexes of such tridentate 2,6-bis(arylimino)pyridyl ligands, bidentate iminopyridyl based ligands were also investigated with first and second row late transition metal (Fe, Co, Ni, Cu, Zn and Pd) halides and some of them have shown exceptional catalytic activities.[2-7] These metal catalyst precursors can be prepared by addition of ligands to either anhydrous or hydrated dihalides in a suitable solvent. Complexes can be isolated as mononuclear species or, depending on both the metal halides and the degree of substitution of the pyridine ring, as centrosymmetric dinuclear molecules.[8-11] By simple modifications of the iminopyridyl architecture, the polymerization and/or oligomerization activities of the metal complexes can be tuned. All these merits make this class of complexes advantageous over other types of metal catalyst, such as metallocenes or geometry constrained species[12-15]. Consequently, synthesis of iminopyridyl based ligands has been a major research target in polymerization catalysis and there have been some studies with asymmetrical iminopyridyl based ligands[9,11,16] and also with long chain aliphatic substituents at the imino nitrogen atom.[17] Additionally, iminopyridyl ligands are suitable synthons in C-C reductive coupling reactions for the synthesis of 1,2-dipyridyl-1,2-diaminoethanes.[18] Recently, some transition metal (Fe(II), Mn(II), Zn(II), Cd(II) or Pd(II)) complexes of unsymmetrical iminopyridyl ligands were found to exhibit atropisomerism, π-π stacking and photoluminescence properties.[19,20]

Zinc is abundant in eukaryotes and plays a variety of physiological roles.[21-23] In addition, Zn(II) complexes are often utilized in many fields such as radioprotection,[24] antidiabetic insulin-mimemetism,[25,26] antibacterial or antimicrobial agents,[27-29] tumour photosensitizers[30] and binder complexes at DNA sites[31-34]. The development of metal chelators for the attenuation of metal-involved neurodegeneration in Alzheimer's disease (AD) and other neurodegenerative diseases are matters of current interest.[35-42] In this perspective, iminopyridyl based zinc(II) complexes were found to be involved in targeting and modulating amyloid-β (Aβ) species which could serve as suitable chemical tools for investigating metal-Aβ-associated events in AD.[41] Furthermore, the use of Zn(II) species as antitumour drugs is very intriguing with respect to existing antitumour therapies;[43] Zn ions are preferred by hydrolytic enzymes due to its redox inertness, low toxicity, hard Lewis acid properties and bioavailability. Hence, efficient Zn(II)-based hydrolytic cleavage agents are good candidates for biomedical applications.[44,45]

In view of the conspicuous importance of the unsymmetrical iminopyridyl systems as metal chelators in catalysis and in biology, we now report the synthesis and full characterization of water soluble zinc(II) compounds of (*E*)-*N*-(pyridin-2-ylmethylene)arylamine (L^n), $[Zn(X)_2(L^n)]$ (X = Cl^-, Br^-, I^-) (**1**- **8**) and $[Zn(\mu\text{-}N_3)(N_3)(L^3)]_2$ (**9**) (Scheme 1), and their potential role as cytotoxic agents. Indeed, the nitrogen environment of the coordination sphere of the zinc compounds coupled with their water solubility are likely to facilitate membrane permeability.[46] Consequently, the dichlorozinc compounds **1**, **3**-**5** and **8** were investigated *in vitro* on HeLa tumour cell lines in order to assess the influence of various substituents and of the metal coordination number on the biological activities.

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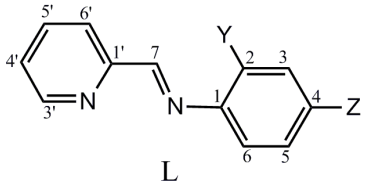
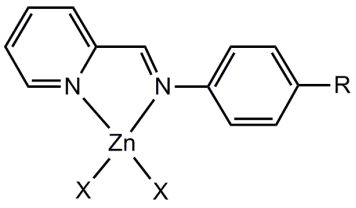
These compounds were characterized by elemental analysis and molar conductance data while their spectroscopic properties were studied using UV-Visible, fluorescence, IR and ^{1}H NMR spectroscopy. Detailed structural information on mononuclear **1** has been obtained by single-crystal X-ray crystallographic analyses by others.[18,41] The molecular structures of the zinc compounds reported here reveal mononuclear structures for **2**-**4, 6**-**8** and binuclear for **9**. In all compounds, L chelates through both nitrogen atoms.



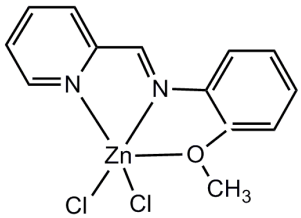
L

L^1 Y = Z = H
L^2 Y = Me, Z = H
L^3 Y = H, Z = Me
L^4 Y = H, Z = CO_2Me
L^5 Y = OMe, Z = H

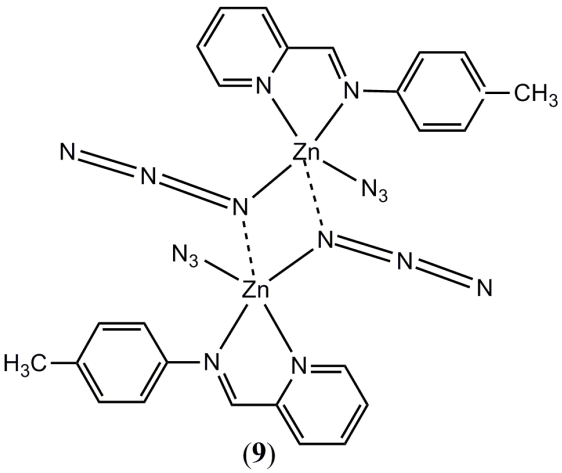
1 $[Zn(Cl)_2(L^1)]$ **5** $[Zn(Cl)_2(L^4)]$ **8** $[Zn(Cl)_2(L^5)]$ **9** $[Zn(\mu\text{-}N_3)(N_3)(L^3)]_2$
2 $[Zn(Br)_2(L^1)]$ **6** $[Zn(Br)_2(L^4)]$
3 $[Zn(Cl)_2(L^2)]$ **7** $[Zn(I)_2(L^4)]$
4 $[Zn(Cl)_2(L^3)]$



(**1**-**4**, **6** and **7**)



(**8**)



(**9**)

Scheme 1. The ligands L^n (n = 1 – 5) with numbering protocol and their zinc(II) compounds **1** – **9**.

Experimental

General considerations.

All chemicals were used as purchased without purification: NaN_3, pyridine-2-carboxaldehyde (Merck), $Zn(OAc)_2$ (Loba-chemie), aniline, $ZnCl_2$ (Sd Fine), $ZnBr_2$, ZnI_2 (Sigma-Aldrich), *o*-toluidine (Thomas Bakers), *p*-toluidine, *o*-anisidine (CDH), *p*-aminobenzoic acid (Hi-Media). *p*-

(carbomethoxyl)aniline (M.p. 113-115 °C) was prepared by reacting a methanol solution of *p*-aminobenzoic acid with $SOCl_2$ under ice-cold conditions followed by a suitable work-up procedure, as described in ref. 47. The ligands (L^1-L^5; Scheme 1) were prepared *in situ* from pyridine-2-carboxaldehyde and the corresponding aniline. Solvents were purified by standard procedures and were freshly distilled prior to use. Melting points were recorded in capillary tubes on a Scanca apparatus and are uncorrected. Elemental analyses were performed using a Perkin Elmer 2400 series II instrument. IR spectra in the range 4000-400 cm^{-1} were obtained using KBr pellets on a Perkin Elmer Spectrum BX series FT-IR spectrophotometer. The ^1H NMR spectra were recorded on a Bruker Avance II spectrometer and measured at 400.13 MHz. The ^1H chemical shifts were referenced to Me_4Si set at 0.00 ppm. Steady-state absorption spectra were recorded at ambient temperature in acetonitrile (spectroscopy grade, Merck) solution on a Perkin-Elmer model Lambda25 absorption spectrophotometer. Fluorescence spectra were obtained on a Hitachi model FL4500 spectrofluorimeter (with the excitation and emission slits fixed at 10 and 20 nm, respectively) and corrected for the instrument response function. Quartz cuvettes of 10 mm optical path length received from PerkinElmer, USA (part no. B0831009) and Hellma, Germany (type 111-QS) were used for measuring absorption and fluorescence spectra, respectively. Fluorescence quantum yields (ϕ_f) were calculated by comparing the total fluorescence intensity over the whole fluorescence spectroscopic range with that of a standard using the method described elsewhere.[48,49] The relative experimental error of the measured ϕ_f was estimated within ±10%. Solution electrical conductivity measurements were made with a Wayne Kerr automatic precision bridge 6440B.

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Synthesis of zinc compounds

The zinc(II) compounds reported here are numbered as **1**-**9** (see Scheme 1) for convenience of discussion. Details of the procedures have been outlined for the syntheses of **1** and **9**. Compounds **2** – **8** were synthesized by following the procedure described for **1**.

Synthesis of $[Zn(Cl)_2(L^1)]$ (**1**). To a solution of pyridine-2-carboxaldehyde (0.172 g, 1.60 mmol) in ethanol (5 mL) was added a solution of aniline (0.15 g, 1.61 mmol) in ethanol (5 mL). The mixture was stirred at ambient temperature for 30 min. To this reaction mixture, $ZnCl_2.2H_2O$ (0.22 g, 1.61 mmol) in methanol (20 mL) was added drop-wise under stirring conditions, which resulted in the immediate formation of a yellow precipitate. The stirring was continued for 3 h and then the mixture was filtered. The residue was washed with methanol (3 x 5 mL) and dried *in vacuo*. The dried solid was dissolved by boiling in 50 mL of acetonitrile and filtered while hot. The filtrate was left for slow evaporation at room temperature affording yellow crystalline material. Yield 0.35 g (64%). M.p. 270-271 °C (M.p. 300 °C (dec.).[18] Anal. Calc. for $C_{12}H_{10}Cl_2N_2Zn$: C, 45.23; H, 3.17; N, 8.80. Found: C, 45.30; H, 3.12; N, 8.88%. Λ_m (CH_3CN): 4 $\Omega^{-1}cm^2mol^{-1}$. IR (cm^{-1}): 1592 ν_{asym}(C(H)=N) + ν(C=N)py; 1498, 1444 ν(C=N)py. ^{1}H-NMR (DMSO-d_6): 8.93 [d, 1H, H-3'], 8.62 [s, 1H, H-7], 8.04 [t, 1H, H-5'], 8.02 [d, 1H, H-6'], 7.63 [t, 1H, H-4'], 7.39-7.20 [m, 5H, H-2,3,4,5,6] ppm. *The spectroscopic data reported in ref. 18 correspond well within the experimental errors.*

Compounds **2** – **9** were prepared as yellow crystals in a manner similar to that described for the preparation of **1**, using appropriate anilines and zinc(II) halides or azides as starting materials.

Synthesis of $[Zn(Br)_2(L^1)]$ (**2**). A similar synthetic procedure as for **1** was used except that $ZnCl_2$ was replaced by $ZnBr_2$, giving pale yellow crystals from acetonitrile solution. Yield 61%. M.p. 318-320 °C. Anal. Calc. for $C_{12}H_{10}Br_2N_2Zn$: C, 35.38; H, 2.47; N, 6.88. Found: C, 35.50; H, 2.52; N, 6.80%. Λ_m (CH_3CN): 5 $\Omega^{-1}cm^2mol^{-1}$. IR (cm^{-1}): 1592ν_{asym}(C(H)=N) + ν(C=N)py; 1498, 1444 ν(C=N)py. ^{1}H-NMR (DMSO-d_6): 9.03 [d, 1H, H-3'], 8.72 [s, 1H, H-7], 8.15 [t, 1H, H-5'], 8.04 [d, 1H, H-6'], 7.76 [t, 1H, H-4'], 7.33 [m, 5H, H-2,3,4,5,6] ppm.

Synthesis of $[Zn(Cl)_2(L^2)]$ (**3**). A similar synthetic procedure as for **1** was used except that aniline was replaced by *o*-toluidine, giving pale yellow crystals from acetonitrile solution. M.p. 269-270 °C. Anal. Calc. for $C_{13}H_{12}Cl_2N_2Zn$: C, 46.95; H, 3.64; N, 8.42. Found: C, 47.10; H, 3.62; N, 8.50%. Λ_m (CH_3CN): 6 $\Omega^{-1}cm^2mol^{-1}$. IR (cm^{-1}): 1634 ν_{asym}(C(H)=N); 1597, 1488, 1444 ν(C=N)py. ^{1}H-NMR (DMSO-d_6): 8.70 [d, 1H, H-3'], 8.50 [s, 1H, H-7], 8.05 [d, 1H, H-6'], 7.97 [t, 1H, H-5'], 7.51 [t, 1H, H-4'], 7.08 [m, 3H, H-2,3,5], 6.88 [d, 1H, H-4], 3.40 [s, 3H,CH_3] ppm.

Synthesis of $[Zn(Cl)_2(L^3)]$ (**4**). A similar synthetic procedure as for **1** was used except that aniline was replaced by *p*-toluidine, giving pale yellow single crystals from acetonitrile solution. Yield: 71%. M.p. 340-345 °C. Anal. Calc. for $C_{13}H_{12}Cl_2N_2Zn$: C, 46.95; H, 3.64; N, 8.42. Found: C, 47.10; H, 3.52; N, 8.50%. Λ_m (CH_3CN): 6 $\Omega^{-1}cm^2mol^{-1}$. IR (cm^{-1}): 1626 ν_{asym}(C(H)=N); 1573, 1460, 1434 ν(C=N)py. ^{1}H-NMR (DMSO-d_6): 8.91 [d, 1H, H-3'], 8.62 [s, 1H, H-7], 8.04 [t, 1H, H-6'], 7.92 [d, 1H, H-5'], 7.65 [t, 1H, H-4'], 7.21 [br m, 2H, H-3,5], 7.07 [d, 2H, H-2,6], 2.25 [s, 3H, CH_3] ppm. *The spectroscopic data reported in ref. 20 correspond well within the experimental errors although the compound was prepared by following a different method.*

Synthesis of $[Zn(Cl)_2(L^4)]$ (**5**). A similar synthetic procedure as for **1** was used except that aniline was replaced by *p*-(carbomethoxyl)aniline giving pale yellow microcrystalline material from acetonitrile solution. Yield: 66%. M.p. 259-260 °C. Anal. Calc. for $C_{14}H_{12}Cl_2N_2O_2Zn$: C, 44.66; H, 3.21; N, 7.44. Found: C, 44.50; H, 3.32; N, 7.50%. Λ_m (CH_3CN): 10 $\Omega^{-1}cm^2mol^{-1}$. IR (cm^{-1}): 1714 ν_{asym}(C=O); 1592 ν_{asym}(C(H)=N) + ν(C=N)py; 1439 ν(C=N)py. ^{1}H-NMR (DMSO-d_6): 9.06 [d, 1H, H-3'], 8.76 [s, 1H, H-7], 8.15 [m, 4H, H-5',6'], 8.02 [m, 2H, H-3,5], 7.75 [t, 1H, H-4'], 7.50 [d, 2H, H-2,6], 3.41 [s, 3H, CO_2CH_3] ppm.

Synthesis of $[Zn(Br)_2(L^4)]$ (**6**). A similar synthetic procedure as for **1** was used except that $ZnCl_2$ and aniline were replaced by $ZnBr_2$ and *p*-(carbomethoxyl)aniline, respectively, giving pale yellow single crystals from acetonitrile solution. Yield: 66%. M.p. 262-265 °C. Anal. Calc. for $C_{14}H_{12}Br_2N_2O_2Zn$: C, 36.13; H, 2.60; N, 6.02. Found: C, 35.90; H, 2.44; N, 6.10%. Λ_m (CH_3CN): 10 $\Omega^{-1}cm^2mol^{-1}$. IR (cm^{-1}):1721 ν_{asym}(C=O); 1591 ν_{asym}(C(H)=N) + ν(C=N)py; 1479, 1445 ν(C=N)py. ^{1}H-NMR (DMSO-d_6): 9.07 [d, 1H, H-3'], 8.80 [s, 1H, H-7], 8.14 [m, 4H, H-5',6'], 7.99 [m, 2H, H-3,5], 7.79 [t, 1H, H-4'], 7.44 [d, 2H, H-2,6], 3.46 [s, 3H, CO_2CH_3] ppm.

Synthesis of $[Zn(I)_2(L^4)]$ (**7**). A similar synthetic procedure as for **1** was used except that $ZnCl_2$ and aniline were replaced by ZnI_2 and *p*-(carbomethoxyl)aniline, respectively, giving pale yellow single crystals from acetonitrile solution. Yield: 51%. M.p. 270-271°C. Anal. Calc. for $C_{14}H_{12}I_2N_2O_2Zn$: C, 30.06; H, 2.16; N, 5.01. Found: C, 30.40; H, 2.10; N, 5.50%. Λ_m (CH_3CN): 4 $\Omega^{-1}cm^2mol^{-1}$. IR (cm^{-1}): 1715 ν_{asym}(C=O); 1588 ν_{asym}(C(H)=N) + ν(C=N)py; 1476, 1432 ν(C=N)py. ^{1}H-NMR (DMSO-d_6): 9.06 [d, 1H, H-3'], 8.87 [s, 1H, H-7], 8.19 [m, 2H, H-5',6'], 8.00 [d, 2H, H-3,5], 7.84 [t, 1H, H-4'], 7.43 [d, 2H, H-2,6], 3.91 [s, 3H, CO_2CH_3] ppm. *Note:*

Results of elemental analysis could not be improved further, yet the purity of the sample was judged on the basis ^{1}H NMR results.

Synthesis of $[Zn(Cl)_2(L^5)]$ (**8**). A similar synthetic procedure as for **1** was used except that aniline was replaced by *o*-anisidine giving pale yellow single crystals from acetonitrile solution. Yield: 59%. M.p. 282-284 °C. Anal. Calc. for $C_{13}H_{12}Cl_2N_2OZn$: C, 44.80; H, 3.47; N, 8.04. Found: C, 44.70; H, 3.62; N, 8.10%. Λ_m (CH_3CN): 5 $\Omega^{-1}cm^2mol^{-1}$. IR (cm^{-1}): 1593 ν_{asym}(C(H)=N) + ν(C=N)py; 1499, 1470 ν(C=N)py. ^{1}H-NMR (DMSO-d_6): 9.25 [s, 1H, H-7], 8.71 [d, 1H, H-3'], 8.10 [m, 2H, H-5',6'], 7.72 [m, 2H, H-4,4'], 7.38 [t, 1H, H-6], 7.05 [m, 2H, 3,5], 4.00 [s, 3H,OCH_3] ppm.

Synthesis of $[Zn(\mu\text{-}N_3)(N_3)(L^3)]_2$ (**9**). To a solution of pyridine-2-carboxaldehyde (0.15 g,1.40 mmol) in ethanol (5 mL) was added a solution of *p*-toluidine (0.15 g, 1.40 mmol) in ethanol (10 mL). The mixture was stirred at ambient temperature for 30 min and was added drop-wise to a stirred methanolic solution containing $Zn(N_3)_2$ (prepared *in situ* from the reaction of $Zn(OAc)_2$ (0.31 g, 1.40 mmol) in 20 mL methanol with an excess of NaN_3 (0.18 g, 2.74 mmol) in 30 mL methanol), which resulted in the immediate formation of a yellow precipitate. The stirring was continued for 3 h and then the mixture was filtered. The residue was washed thoroughly with water, then with methanol (3 x 5 mL) and dried *in vacuo*. The dried solid was dissolved by boiling in 60 mL of acetonitrile and filtered while hot. The filtrate, upon cooling to room temperature, afforded yellow crystalline material. Yield 0.29 g (47%). M.p. 222-225 °C. Anal. Calc. for $C_{26}H_{24}N_{16}Zn_2$: C, 45.17; H, 3.50; N, 32.42. Found: C, 45.30; H, 3.10; N, 32.66%. Λ_m (CH_3CN): 9 $\Omega^{-1}cm^2mol^{-1}$. 1630 ν_{asym}(C(H)=N); 1591; 1475, 1441 ν(C=N)py; 2039 $\nu_{as}(N_3)$. ^{1}H-

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NMR (DMSO-d_6): 8.82 [d, 1H, H-3'], 8.70 [s, 1H, H-7], 8.09 [m, 2H, H-5',6'], 7.65 [t, 1H, H-4'], 7.23 [d, 2H, H-3,5], 7.18 [d, 2H, H-2,6], 2.35 [s, 3H, CH_3] ppm. *Note: While no incident occurred while using azide during preparation and isolation, care in handling azides must be exercised owing to their potentially explosive nature.*

X-ray crystallography

Crystals of zinc compounds (**2**-**4, 6**-**9**) suitable for an X-ray crystal-structure determination were obtained by slow evaporation of acetonitrile solutions of the respective compounds at room temperature. The data for **2, 4, 6, 7** and **9** were recorded at low temperature on an Agilent Technologies SuperNova area-detector diffractometer[50] using Mo Kα radiation (λ = 0.71073 Å) from a micro-focus X-ray source and an *Oxford Instruments Cryojet XL* cooler. The measurements for **3** and **8** were made at low temperature on a Nonius KappaCCD diffractometer[51] with graphite-monochromated Mo Kα radiation (λ = 0.71073 Å) and an Oxford Cryosystems Cryostream 700 cooler.

Data reduction on **3** and **8** were performed with HKL Denzo and Scalepack[52] while CrysAlisPro[50] was used for **2, 4, 6, 7** and **9**. The intensities were corrected for *Lorentz* and polarization effects. Empirical absorption corrections based on the multi-scan method using spherical harmonics[53] were applied. Equivalent reflections were merged. The data collection and refinement parameters are given in Table 1, and perspective views of the structures are shown in Figs. 2-4. The structures were solved by direct methods using *SHELXS97*[54] which revealed the positions of all non-hydrogen atoms. The largest peak of residual electron density in **2** is within 0.90 Å of the Zn-atom. In **9**, the molecule is a dinuclear Zn-complex with a crystallographic centre of inversion located in the middle of the central Zn_2N_2 core. The non-hydrogen atoms in

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each structure were refined anisotropically. All of the H-atoms were placed in geometrically calculated positions and refined by using a riding model where each H-atom was assigned a fixed isotropic displacement parameter with a value equal to $1.2U_{eq}$ of its parent atom ($1.5U_{eq}$ for any methyl group). The refinement of each structure was carried out on F^2 by using full-matrix least-squares procedures, which minimized the function $\Sigma w(F_O^2–F_C^2)^2$. A correction for secondary extinction was applied for **3** and **6**-**8**. Two reflections in **8**, whose intensities were considered to be extreme outliers, were omitted from the final refinement. The *SHELXL97* program[54] was used for all refinements**.**

Isolation of peripheral blood mononuclear cells (PBMC) from human blood

PBMC were separated from fresh peripheral human blood of normal healthy donors by the ficoll-paque (Histopaque-1077) density gradient centrifugation method. The oxalated blood was immediately diluted with sterile 1:1 phosphate buffer saline (PBS), layered over ficoll paque (1.077 density) and centrifuged at 400 xg for 30 minutes at room temperature. The PBMC layer formed above the ficoll level was removed by aspiration and suspended in PBS, centrifuged and the pellet suspended in RPMI medium for culture. The viability of the cells was checked with the trypan blue exclusion test: the viable cells exclude the dye and the non-viable cells take-up the dye giving a blue coloration. The diluted cell suspension was taken in 0.2% trypan blue dye in saline and dye positive and negative cells were counted separately under a microscope using Hemocytometer, and the amount expressed in percentage. The cells were found to be 98% viable by the trypan blue dye exclusion test.

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Experimental protocols for MTT, LDH and DNA binding assays

The drug-induced cytotoxicity was measured by the MTT assay.[55] Briefly, 50,000 HeLa cells per well (24 well plate) or 5000 PBMC per well (96 well plate) were incubated in the presence or absence of the indicated test samples (zinc compounds: **1**, **3-5** and **8**) and cisplatin in a final volume of 1 mL for 72 h at 37 °C in duplicate. Then, 0.025 mL of MTT solution (5 mg/mL in Phosphate buffered saline (PBS)) was added to each well. After 2 h of incubation at 37 °C, 0.5 mL of extraction buffer (20% sodium dodecyl sulfate (SDS) in 50% dimethlylformamide) was added. After an overnight incubation at 37 °C, the absorbance at 570 nm was measured using a 96 well multiscanner auto reader (Coda, Bio Rad) with the extraction buffer as blank. Mean absorbance was taken and cell death was calculated considering untreated cells viability as 100%.

The cytosolic marker lactate dehydrogenase (LDH) enzyme was assayed from the culture supernatant[56] from zinc compounds-treated cells. Cells were treated with different concentrations of zinc compounds **1**, **3-5** and **8** for 72 h, and the culture supernatants were collected and incubated with substrate solution (0.23 M sodium pyruvate and 5 mM NADH in 0.1 M phosphate buffer, pH 7.5). The absorbance was recorded at 420 nm after 30 min of incubation at 37 °C.

In vitro DNA binding assays were performed with HeLa cells which were cultured in 60 mm petri dishes overnight and stimulated with 1 nM of tumour necrosis factor (TNF) for 1 h. The nuclear extracts (NE) were prepared and 15 µg of NE proteins were taken for *in vitro* DNA binding assay.[57] NE were incubated with different concentrations (see above) of zinc compounds **1**, **3-5** and **8**, for 30 min at 37 °C and then transcription factors NF-κB and AP-1, and p53 DNA binding were assayed using ^{32}P-labelled specific gel shift double stranded oligonucleotides at 6%

native PAGE. The gels were dried, exposed to Phosphorimager screen overnight, scanned and analysed in Phosphorimager (Fuji, Japan).

Gel shift oligonucleotides:

NF-κB: 5'-TTG TTA CAA GGG ACT TTC CGC TGG GGA CTT TCC AGG GAG GCG TGG-3'

AP-1: 5'-CGC TTG ATG ACT CAG CCG GAA-3'

p53: 5'-TAC AGA ACA TGT CTA AGC ATG CTG GGG ACT-3'

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Table 1 Crystal data and refinement details[a] for zinc compounds **2-4, 6-9**

	2	**3**	**4**
Empirical formula	$C_{12}H_{10}Br_2N_2Zn$	$C_{13}H_{12}Cl_2N_2Zn$	$C_{13}H_{12}Cl_2N_2Zn$
Formula weight	407.41	332.54	332.51
Crystal size (mm)	0.05 × 0.17 × 0.20	0.12 × 0.20 × 0.28	0.14 × 0.15 × 0.25
Crystal morphology	Tablet	Prism	Prism
Temperature (K)	160(1)	160(1)	160(1)
Crystal system	Triclinic	Monoclinic	Monoclinic
Space group	$P\bar{1}$	$P2_1/c$	$P2_1/n$
a (Å)	7.8621(3)	13.8601(2)	11.7947(3)
b (Å)	8.8368(3)	8.2826(1)	9.78427(18)
c (Å)	10.0742(3)	12.6858(2)	12.0762(2)
α (°)	102.598(3)	90	90
β (°)	100.816(3)	105.682(1)	104.121(2)
γ (°)	96.996(3)	90	90
V (Å^3)	661.19(4)	1402.09(4)	1351.51(5)
Z	2	4	4
D_x (g cm^{-3})	2.046	1.575	1.634
μ (mm^{-1})	7.897	2.115	2.194
Transmission factors (min, max)	0.534, 1.000	0.641, 0.773	0.862, 1.000
θ range (°)	2.12–29.42	2.89–30.03	2.71–30.37
Reflections measured	14700	40741	17244
Independent reflections; R_{int}	3307; 0.041	4100; 0.045	3766; 0.023
Reflections with $I > 2\sigma(I)$	2810	3463	3340
Number of parameters	154	165	164
$R(F)$ [b] [$I > 2\sigma(I)$reflns]	0.0346	0.0318	0.0275
$wR(F^2)$ [c] (all data)	0.0777	0.0824	0.0725
GOF(F^2)	1.039	1.031	1.134
$\Delta\rho_{max, min}$ (e, Å^{-3})	2.86, -1.05	1.13, -0.50	0.65, -0.30

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Table 1 contd.

6	**7**	**8**	**9**
$C_{14}H_{12}Br_2N_2O_2Zn$	$C_{14}H_{12}I_2N_2O_2Zn$	$C_{13}H_{12}Cl_2N_2OZn$	$C_{26}H_{24}N_{16}Zn_2$
465.45	559.45	348.54	691.34
0.18 × 0.20 × 0.25	0.10 × 0.18 × 0.22	0.10 × 0.15 × 0.43	0.10 × 0.12 × 0.22
Prism	Prism	Tablet	Prism
160(1)	160(1)	160(1)	160(1)
Monoclinic	Monoclinic	Monoclinic	Monoclinic
*C*2/*c*	*C*2/*c*	$P2_1/n$	$P2_1/c$
17.6449(3)	18.3886(2)	7.6346(1)	8.69797(13)
13.1494(2)	13.12663(16)	18.9972(4)	17.7362(3)
13.8486(2)	14.11370(17)	9.7619(1)	9.75808(16)
90	90	90	90
99.4538(15)	100.1521(12)	94.8419(12)	107.3946(17)
90	90	90	90
3169.51(9)	3353.44(7)	1410.77(4)	1436.53(4)
8	8	4	2
1.951	2.216	1.641	1.598
6.612	5.153	2.111	1.720
0.591, 1.000	0.620, 1.000	0.627, 0.816	0.885, 1.000
2.33–29.54	2.25– 32.37	2.14−30.02	2.30−30.27
18117	26438	33068	18231
4048; 0.037	5627; 0.026	4118; 0.048	3948; 0.023
3542	5061	3469	3516
192	192	174	200
0.0264	0.0192	0.0287	0.0246
0.0570	0.0439	0.0720	0.0598
1.039	1.053	1.054	1.079
0.58, -0.66	0.75, -0.60	0.54, -0.64	0.33, -0.33

[a]Data for **1** has been reported in refs. 18 and 41.

[b] $R1 = \Sigma||F_o| - |F_c||/\Sigma|F_o|$.

[c] $wR2 = [\Sigma[w(F_o^2 - F_c^2)^2]/ \Sigma[w(F_o^2)^2]]^{1/2}$

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Results and discussion

Syntheses

Zinc compounds **1**-**9** were prepared by one-pot reaction of ZnX_2 (X = halides or azide) with L^n (generated *in situ* from pyridine-2-carboxaldehyde and a substituted aniline), akin to that reported for mercury analogues.[48,49] The general stoichiometries of the $[Zn(X)_2(L^n)]$ complexes have been corroborated by the microanalytical results, which clearly evidence the formation of 1:1 adducts between the bidentate *N*-donors L^n and ZnX_2. In all cases, air stable pale yellow solids were obtained in moderate to good yields and these compounds behave as non-electrolytes in acetonitrile solution. Compounds **1**-**9** are soluble in water, alcohol, acetonitile, DMSO and DMF. The spectroscopic properties were studied using UV-Visible, fluorescence, IR and ^{1}H NMR spectroscopy while the solid state structures of **2**-**4** and **6**-**9** were determined by single crystal X-ray crystallography.

IR, NMR, UV-Vis and fluorescence spectroscopy

The IR spectra of **1**-**9** exhibit moderately intense bands in the 1590-1620 cm^{-1} range which were assigned to the ν_{asym}(C(H)=N) stretch of the coordinated Schiff base ligands.[48,49,58] In addition, well resolved-sharp bands of variable intensity observed in the 1600-1580, 1490-1475 and 1450-1435 cm^{-1} regions were assigned to the coordinated pyridine ring.[48,49,58-60] In **9**, it is worth mentioning that the very strong band at 2037 cm^{-1} corresponds to $\nu_{as}(N_3)$,[61,62] a value similar to that recently reported for $\{[Hg(N_3)_2L]_2\}_n$.[48] The ^{1}H NMR spectra of the compounds in DMSO-d_6 solution displayed the expected signals, which correlate well with the hydrogen atoms present in the molecules. Coupling constants could not be determined with certainty owing to the broad unresolved nature of the signals.[48,49]

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Table 2 Photophysical data for compounds **1**-**9** in acetonitrile solution

Compounds	Electronic spectroscopic data λ_{max} (nm); (ε[$M^{-1}cm^{-1}$])	Photoluminescence data λ_{em} (nm)[a]	ϕ_F
1	328 (13,191)	412, 430	0.34
2	329 (26,190)	411, 431	0.06
3	334 (21,329)	414, 430	0.16
4	342 (14,260)	413, 430	0.19
5	328(14,150)	411, 432	0.09
6	327 (12,228)	414, 427	0.04
7	325(26,682)	412, 429	0.11
8	325(10,174), 371(11,308)	413, 431, 502	0.13
9	340(11,720)	410, 426	0.08

[a]The long wavelength emission appears as a shoulder in all the cases.

Table 2 summarizes the solution UV-Vis and fluorescence properties of compounds **1-9**. The absorption spectra of all compounds were recorded in the 300-450 nm range in acetonitrile solutions at concentrations of ~10^{-5} M; they generally comprise one intense band, except for **8** where two overlapping bands were detected (Fig. 1a). The electronic spectra of the ligands are not available. In general, the zinc compounds displayed a broad fluorescent emission band at λ_{em} = 410 nm (Fig. 1b) along with a shoulder in the range ~426-432 nm at room temperature. The *o*-methoxy derivative **8** displayed an additional band at 502 nm. These emissions could not be assigned as metal-to-ligand charge transfer (MLCT) or ligand-to-metal charge transfer (LMCT) as the zinc(II) ion, with a d^{10} configuration, is not easily oxidized or reduced.[63] The emissions are attributed to the intraligand (IL) (π–π^*) fluorescent emission. On the whole, the Zn compounds

show a very low fluorescent quantum yields (Table 2), indicating that they are weak emitters at room temperature.

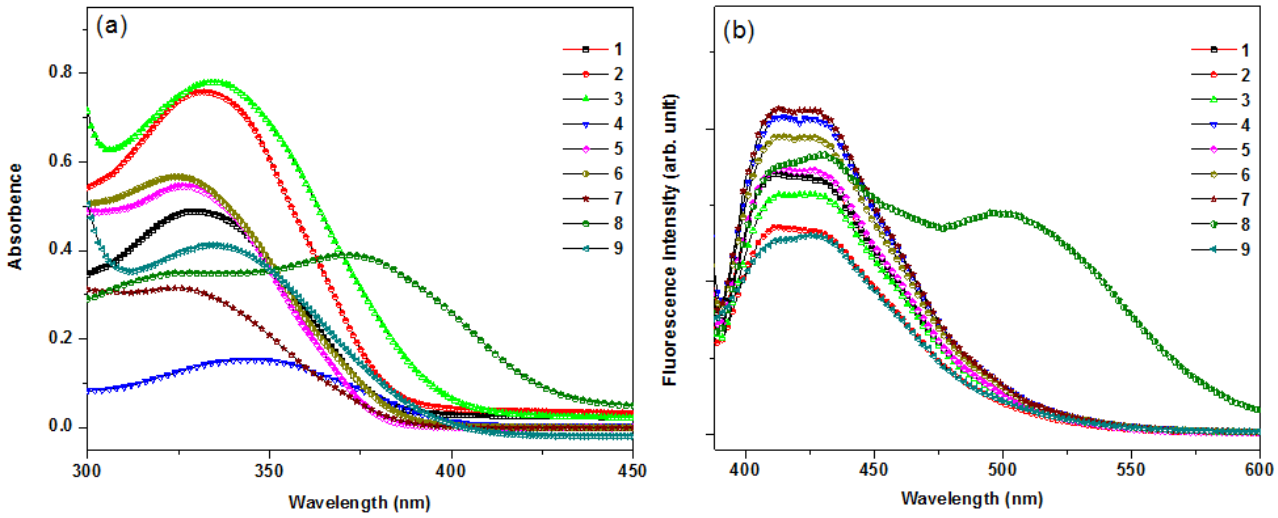

Fig. 1 (a) UV–Vis spectra of compounds **1-9** in acetonitrile (concentration ~10^{-5} M) (b) Fluorescence spectra of compounds **1-9** in acetonitrile (concentration ~10^{-5} M) obtained by excitation at the respective absorption maxima.

Molecular structures

Although a substantial amount of work has been reported with analogous L ligands (see Introduction), to our knowledge the extant literature contains no crystallographic characterization of L except for the one described by Wiebcke *et al.* and that too suffers from whole molecule disorder.[64] In view of this and to assign the diagnostically important experimental infrared bands, quantum chemical calculations were carried out on some of these ligands and their structural parameters were reported earlier by us.[48] The crystal structure of compound **1** has been reported by others on two different occasions.[18,41] Recently, the structure of **1** has also been validated by ground state optimization by various DFT methods.[65] The results of the crystal structure determinations of zinc compounds **2-4** and **6-9**, now reported, are consistent with the chemical

and spectroscopic analyses. The molecular structures of **2**-**4**, **6** and **7** are displayed in Fig. 2 and those of **8** and **9** in Fig. 3 and Fig. 4, respectively. Generally, the zinc atom prefers a tetrahedral environment; however, depending on the bulkiness of the ligands, coordination numbers between two and six can be realized. In **2**-**4**, **6** and **7**, the Zn cations are in a tetrahedral coordination environment, with τ_4 values[66] in the range 0.83 – 0.89 (Table 3). However in **8**, the Zn cation is in a distorted square pyramidal environment ($\tau_5 = 0.19$)[67] with the basal plane defined by atoms Cl2, O1, N1 and N2 and the apical position occupied by atom Cl1. The molecule of [Zn(μ-N_3)(N_3)(L^3)]$_2$ (**9**) is binuclear and sits across a crystallographic centre of inversion. The coordination sphere of the symmetry-unique zinc atom is trigonal bipyramidal ($\tau_5 = 0.67$)[67] with atoms N1, N3 and N6 in the equatorial positions and the axial sites occupied by atoms N2 and $N6^i$ (Fig. 4). In all the compounds, both imino-N and pyridine-N atoms are involved in the chelation, leading to the formation of at least one metallacycle five-membered ring. In addition, a second five-membered metallacycle is found in **8** owing to the coordination of the methoxy O-atom to zinc (Fig. 3), while in **9**, a four-membered ring is present in the central Zn_2N_2 core (Fig. 4).

As a result of imposed steric constraints, either from a non-chelating *ortho*- methyl substituent on the phenyl ring (as in **3**) or from packing interactions (as in **6** and **7**), the organic ligands in some complexes deviate significantly from a planar conformation. This is reflected in the Zn1–N2–C7–C12 torsion angles (absolute values range from 6.6(3) to 69.4(2)º; Table 3) and also in the dihedral angles between the planes of the phenyl and Zn-N-C-C-N metallacyclic rings which adopt values from 7.20(16)° (in **2**) to 64.11º (in **3**). The organic chelating groups in **2**, **4** and **8** are the most planar with the Zn1–N2–C7–C12 torsion angles and the corresponding dihedral angles all being less than 10° (Table 3). In compound **9**, these two parameters adopt

intermediate values, while compound **3** is the most twisted case on account of the *ortho*- methyl substituent. In all the compounds, the N1–C6 bond distances are in the range 1.275(4) – 1.284(2) Å, which confirms the double-bond character of the methylidene groups. In general, the metal-$N_{pyridine}$ (Zn1–N1) bond distances are shorter than the metal-$N_{methylidene}$ (Zn1–N2) lengths (Table 3), the exception arising only in **8** probably as a result of a certain amount of *trans* effect from the coordinated methoxide group (O1–Zn1–N1 = 146.07(5)º). In **9**, the terminal Zn-N_{azide} bond lengths (1.9799(13) Å) are significantly shorter than those involving the asymmetrically bridging azide (2.0414(12), 2.1918(12) Å) and the two zinc atoms of the central Zn_2N_2 unit are 3.2517(2) Å apart.

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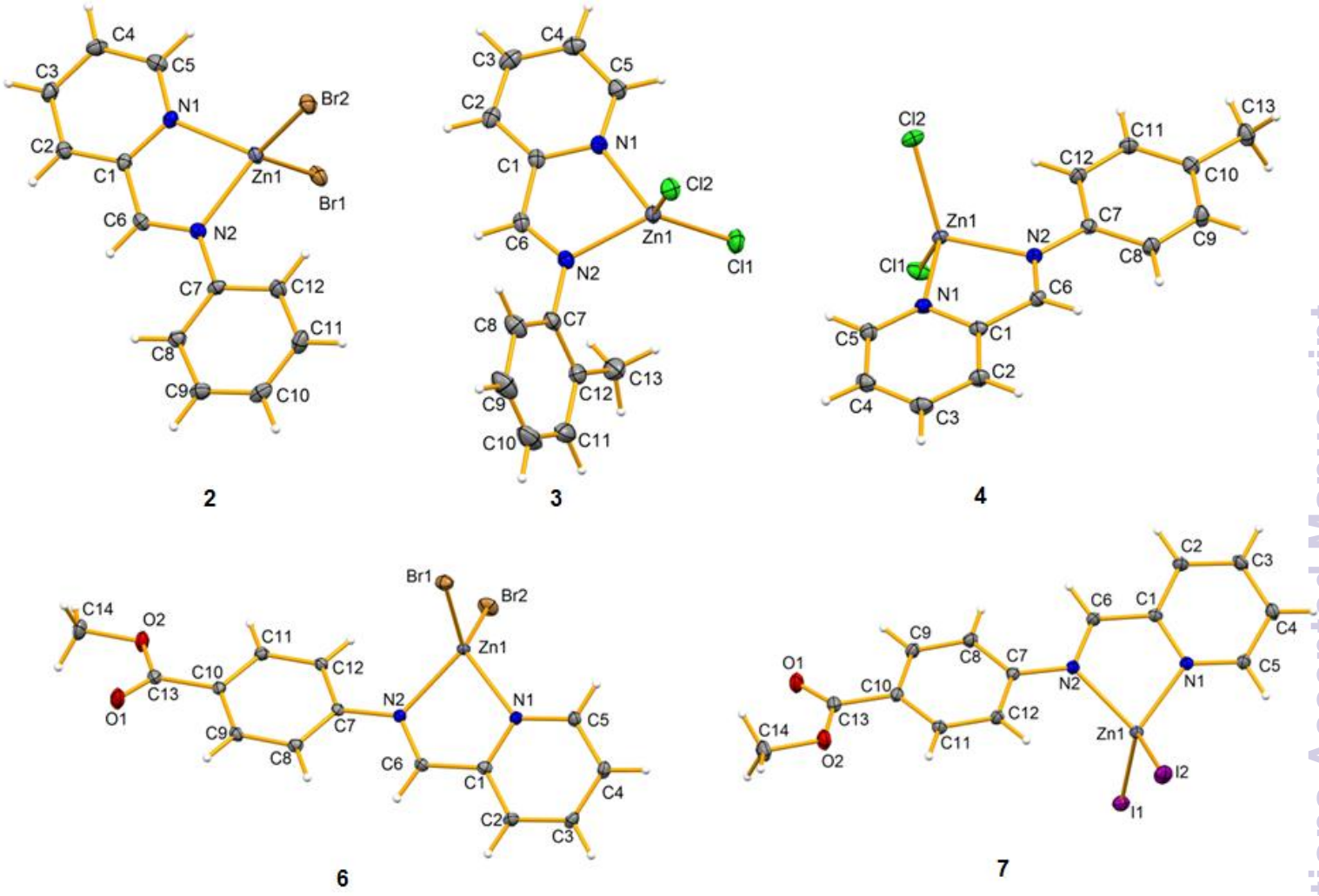

Fig. 2 Perspective views of the molecular structures of $[Zn(Br)_2(L^1)]$ (**2**), $[Zn(Cl)_2(L^2)]$ (**3**), $[Zn(Cl)_2(L^3)]$ (**4**), $[Zn(Br)_2(L^4)]$ (**6**) and $[Zn(I)_2(L^4)]$ (**7**) with the atomic numbering schemes. Displacement ellipsoids are drawn at the 30% probability level and H atoms are shown as small spheres of arbitrary radii.

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Table 3 Zn coordination geometry and selected geometrical parameters for the zinc compounds **2-4** and **6-9**

	2	**3**	**4**	**6**	**7**	**8**	**9**
Geometry[a]	Tet	Tet	Tet	Tet	Tet	Spy	Tbp
Coordination sphere	N_2Br_2	N_2Cl_2	N_2Cl_2	N_2Br_2	N_2I_2	N_2Cl_2O	N_5
Geometry indices, τ_4 (τ_5)	0.88	0.83	0.89	0.89	0.88	(0.19)	(0.67)
Zn1–N1 (Å)	2.063(3)	2.0608(15)	2.0558(16)	2.0475(17)	2.0454(14)	2.1313(15)	2.0735(11)
Zn1–N2 (Å)	2.086(3)	2.0755(15)	2.0782(15)	2.0960(17)	2.0992(14)	2.1042(13)	2.1945(12)
N2–C6 (Å)	1.275(4)	1.277(2)	1.284(2)	1.282(3)	1.283(2)	1.280(2)	1.2759(18)
N1–Zn1–N2 (°)	80.81(11)	80.59(6)	81.17(6)	80.71(7)	80.52(5)	77.82(5)	77.62(4)
Zn1–N2–C7–C12 (°)	6.6(4)	-69.4(2)	8.0(2)	27.4 (3)	27.8(2)	9.35(19)	14.13(18)
Metallacycle/phenyl plane dihedral angle (°)	7.20(16)	64.11(10)	8.71(9)	26.28(10)	27.01(7)	7.23(8)	17.43(7)
Shortest Zn···Zn (Å)	6.2723(7)	5.6040(3)	6.4256(3)	5.5039 (3)	5.7174(3)	5.3058(3)	7.6437(3)

[a] Tet = Tetrahedral; Spy = Square pyramidal and Tbp = Trigonal bipyramidal.

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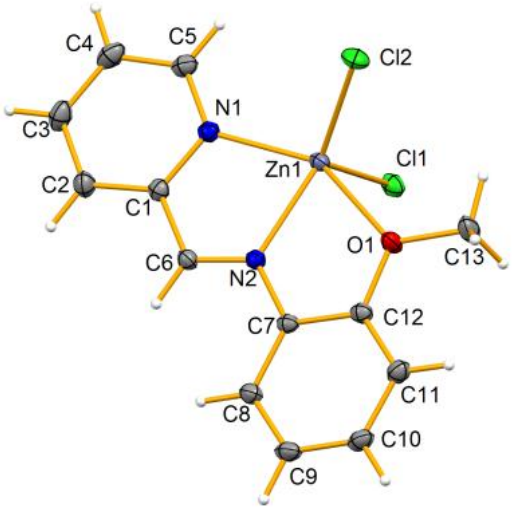

Fig. 3 Perspective view of the molecular structure of $[Zn(Cl)_2(L^5)]$ (**8**) with the atomic numbering scheme. Displacement ellipsoids are drawn at the 30% probability level and H atoms are shown as small spheres of arbitrary radii.

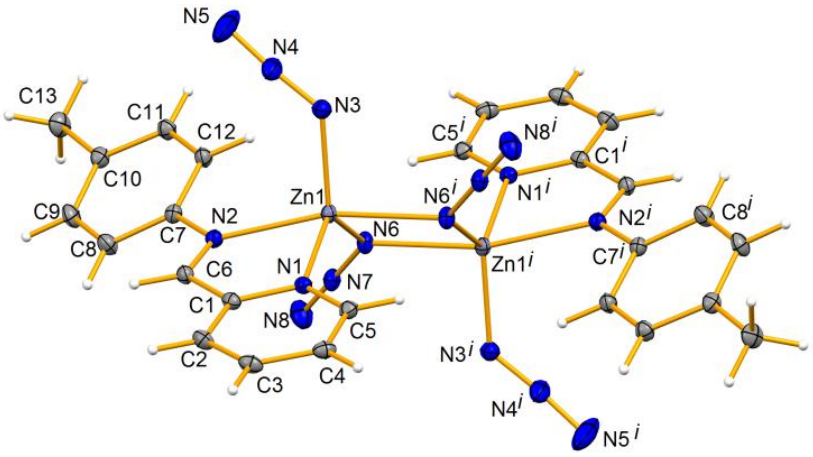

Fig. 4 Perspective view of the two centrosymmetrically-related azide-bridged units, found in the crystal structure of $[Zn(\mu\text{-}N_3)(N_3)(L^3)]_2$ (**9**) with the atomic numbering scheme. Displacement ellipsoids are drawn at the 30% probability level and H atoms are shown as small spheres of arbitrary radii.

Non-covalent non-classical intermolecular interactions are present in all the compounds, particularly in the case of compounds **6** and **7**, where the ester moieties act as medium-strong acceptors of $C_{phenyl}H\cdots O$ interactions [*d*(D···A) in the range 2.973(3) – 3.062(2) Å and ∠(D–H···A) between 113 and 118º] giving rise to infinite one-dimensional chains running parallel to the crystallographic *b* axis (Fig. S1 and S2). In the other compounds, such interactions involve the halogen atoms (or the terminal nitrogen atom of the non-bridging azide in **9**) as the $C_{phenyl}H$ acceptors [*d*(D···A) distances longer than 3.315(3)Å]. In this respect, complex **3** is a good example (Fig. S2): every molecule is surrounded, only by means of such contacts, by eight other molecules and in this cluster the shortest Zn···Zn distance is 5.6040(3) Å.

Intermolecular π···π stacking interactions have been found in these structures, with some of their values suggesting a perfect (face-to-face), or slightly offset stack ring *centroid···centroid* distances values typically fall in the range of 3.3 – 3.8 Å.[68] Indeed, the one-dimensional chains in **6** are assembled by such interactions which involve the phenyl rings of adjacent molecules (*centroid···centroid* = 3.6782(13) Å, Fig. S1, top); in **7** such contacts are weaker (3.8871(10) Å, Fig. S1, bottom). The shortest *centroid···centroid* contacts of 3.5175(9) and 3.6523(11) Å were found for, respectively, the intermolecular interactions involving the pyridine rings of adjacent molecules in **9** (Fig. S3) and the phenyl ring in one molecule and the pyridine moiety in a vicinal molecule in **4** (Fig. S4). Such interactions assemble these structures into chains which run parallel to the crystallographic *c* axis in **9** or in a zig-zag fashion along the *b* axis in **4**. The minimum Zn···Zn distances in **9** and **4** are 7.6437(3) and 6.4256(3) Å, respectively. In **2**, 3.674(2) Å is the *centroid···centroid* distance for the shortest intermolecular π···π interaction, which involves the metallacycle and the phenyl ring of one molecule interacting with the phenyl ring and the metallacycle, respectively, of an adjacent molecule (Fig. S5). By means of such

interactions, the molecules of **2** appear to be associated in pairs, which then interact with neighbouring pairs through pyridine···phenyl π···π contacts of 3.872(2) Å, resulting in stacks of molecules arranged parallel to the crystallographic *a* axis. The Zn···Zn distance within these pairs of molecules (7.4792(7) Å) is considerably greater than the Zn···Zn distance between pairs in the same stack (6.2723(7) Å, Table 3). Also in **4** and in **8** there are π···π interactions linking the metallacyclic rings, yet of weaker intensity (*centroid···centroid* distances in the range 3.8035(10) – 3.9501(8) Å); together with **2**, these compounds present the flattest L ligands found in this study (Table 3).

Biological evaluation

To elucidate the mechanism of cytotoxic potency, we have selected the five dichlorozinc derivatives **1**, **3**-**5** and **8**, see **Scheme 1**, whose molecules satisfy the restrictive terms of Lipinski's rules (low molecular weight (MW ≤ 450); relatively lipophilic (clogP, calculated logarithm of the octanol/water partition coefficient, ≤5); hydrogen-bond donor atoms (HBD ≤ 5); hydrogen-bond acceptor atoms (HBA ≤ 10); small polar surface area (PSA ≤ 90 $Å^2$)), criteria essential for the rational structure-based drug design.[69,70] The molecules of **1**, **3**-**5** and **8** are small (MW ≤ 377) and their solubility in water ultimately makes them biocompatible, which is likely to facilitate their biological application, and has prompted us to engineer these compounds as potential anticancer agents. Following rational structure-based design principles, the cytotoxicity activities of **1**, **3**-**5** and **8** have been investigated *in vitro* toward the human epithelial cervical carcinoma (HeLa) cell lines and the apoptosis-inducing activities were assessed by MTT, LDH and DNA binding assays.

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MTT, LDH and DNA binding assays

HeLa cells were incubated with different concentrations of the zinc compounds and cisplatin and the cell viabilities were assayed by the MTT method. The zinc compounds increased the cell death in a concentration-dependent manner (Fig. 5). The IC_{50} values of the test compounds and cisplatin were calculated from the data obtained from MTT assays and are collected in Table 4. The cytotoxic effect of cisplatin was considered as the positive control for this experiment.

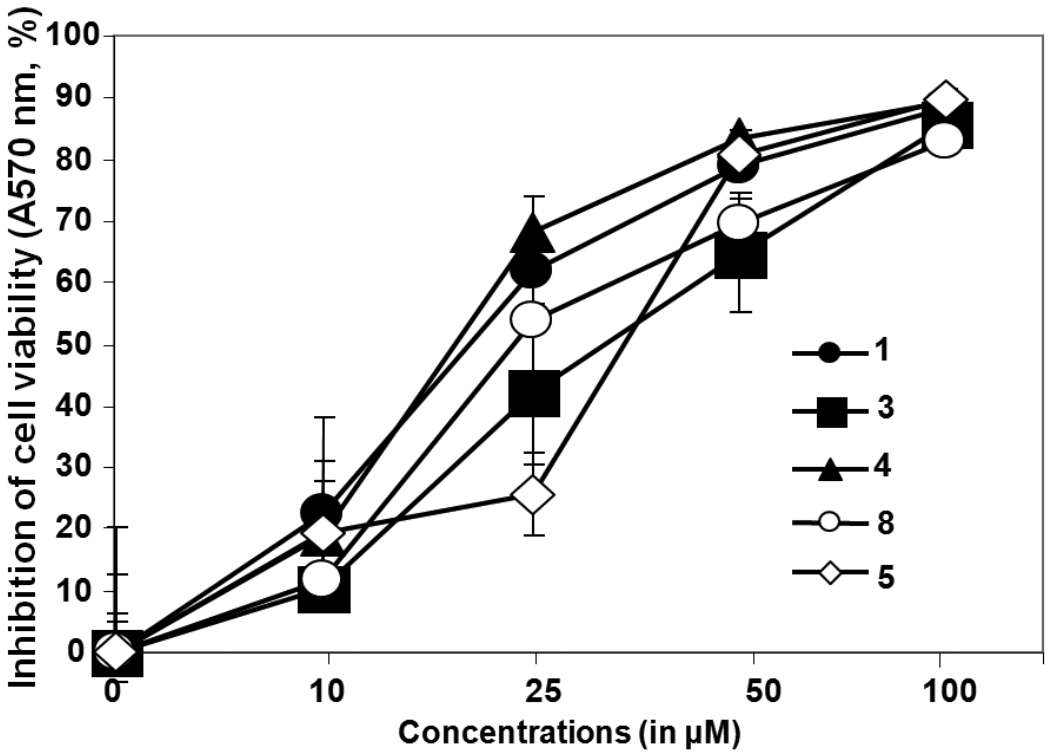

Fig. 5 Effect of zinc compounds on cell viability. HeLa cells (50,000/well of 24-well plate) were treated with different concentrations of zinc compounds **1**, **3**, **4**, **5** and **8** for 72 h in duplicate. For positive control, cisplatin was used for the same assay. Cells were used for MTT assays. The plate was kept overnight at 37 $^{\circ}$C and the absorbance was recorded at 570 nm. The cell death was calculated from duplicate samples and indicated as inhibition of cell viability in percentage

considering untreated cells as 100% viable cells. The experiment was repeated twice and the data from one experiment is represented.

Table 4 *In vitro* IC_{50} values (μM)[a] of test zinc compounds **1**, **3**-**5** and **8** and the standard drug (cisplatin = CDDP) in HeLa cell line

Test compounds/ Standard drug	IC_{50} (μM)
$[Zn(Cl)_2(L^1)]$ (**1**)	19.5 (± 0.5)
$[Zn(Cl)_2(L^2)]$ (**3**)	28.25 (± 1.25)
$[Zn(Cl)_2(L^3)]$ (**4**)	17.75 (± 0.25)
$[Zn(Cl)_2(L^4)]$ (**5**)	33.6 (± 1.1)
$[Zn(Cl)_2(L^5)]$ (**8**)	20.82 (± 2.32)
CDDP	0.36

[a] The values are calculated from the data obtained by MTT assay, as shown in Fig. 5. The standard drug reference value is cited under identical conditions. The standard deviation (in brackets) is calculated from the data obtained from two independent experiments.

The molecules of zinc compounds **1**, **3**-**5** and **8** are small (MW ≤ 377) and generally small molecules are known to exert several side effects including cytolysis and hence the LDH assay was performed. HeLa cells and blood-derived PBMC were incubated with different concentrations of zinc compounds **1**, **3**-**5** and **8** for 72 h and the culture supernatant was used in the measurement of LDH. No significant increase in the activity of LDH was observed for the cells treated with zinc compounds even up to 100 μM concentration for 72 h (Figs. S6 and S7). The primary cells (PBMC) treated with the zinc compounds did not show any significant cell death and cytolysis, as determined by the MTT and LDH assays, respectively.

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In vitro DNA binding assay of zinc compounds **1**, **3**-**5** and **8** was performed with HeLa cells. The binding of nuclear transcription factor kappaB (NF-κB), activator protein 1 (AP-1), and p53 DNA were determined. Nuclear extracts were incubated with different concentrations of zinc compounds and DNA binding was assayed using specific double stranded oligonucleotides. The DNA binding was found to decrease with increasing concentration of zinc compounds **1**, **3**-**5** and **8** for NF-κB, AP-1 and p53 (Fig. 6). The data suggest that the zinc compounds interfere with DNA-protein binding, but only for the two higher concentrations (15 and 30 μM).

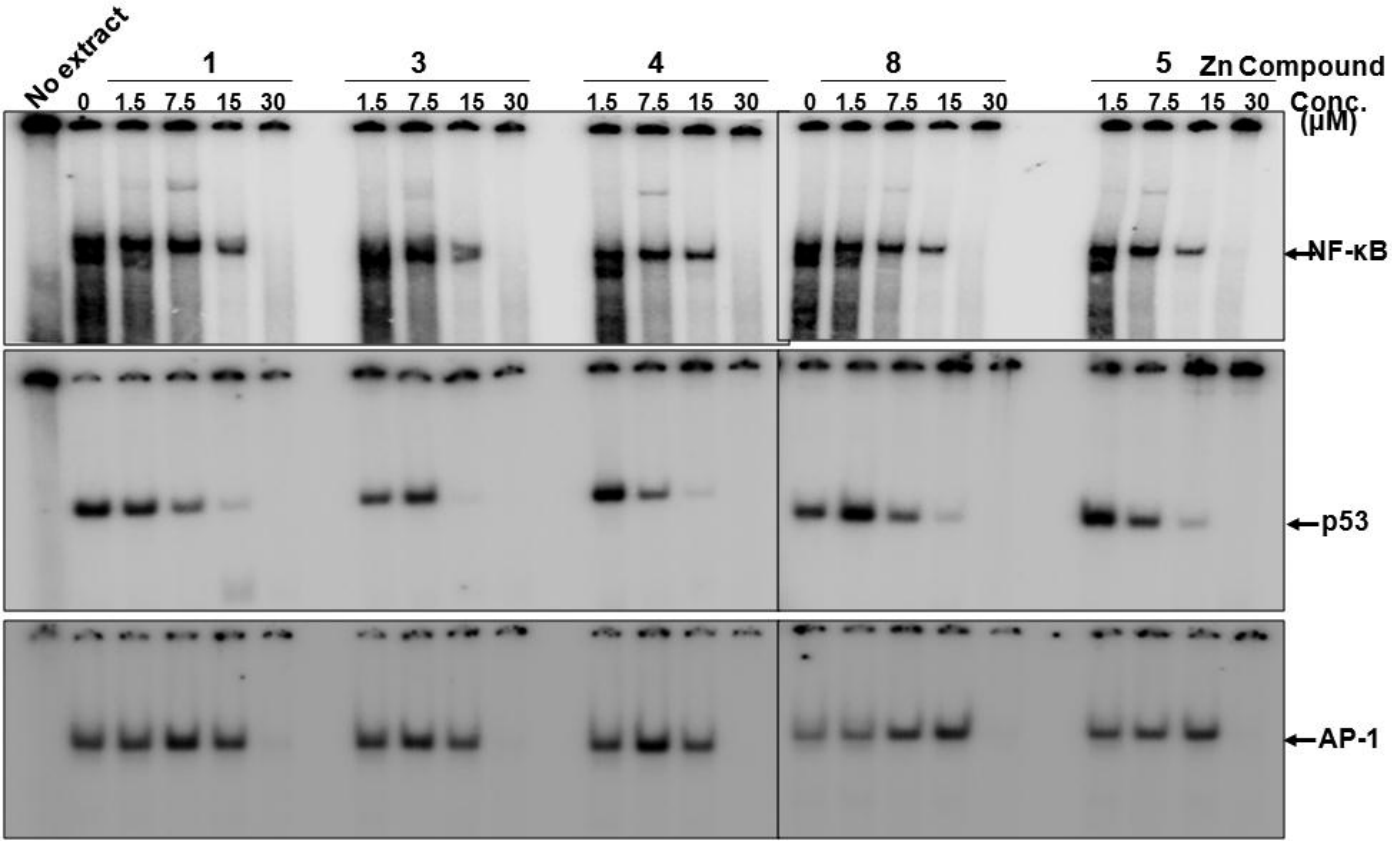

Fig. 6 The effect of zinc compounds **1**, **3**-**5** and **8** on DNA binding activity. HeLa cells were used to isolate the nuclear extracts. The nuclear extracts (15 μg protein) were incubated with different concentrations of zinc compounds for 30 min at 37 °C and then the DNA binding activity for NF-κB, AP-1 and p53 was determined by gel shift assay.

Live cells show oxidative burst response that signifies their viability as these cells utilize oxygen for biological reactions that need energy for survival. Tetrazolium dye (MTT) converted to formazan granules upon exposure to reactive oxygen, and their formation was measured a as marker for cell viability. Treatment of HeLa cells with the zinc compounds **1**, **3**-**5** and **8** shows moderate cell death indicating potent anti-tumour activity. Although these compounds increase cell death, cytolysis was not observed even at higher concentrations, indicating the safe use of these compounds for therapy.

Whether these zinc compounds are specific to tumour or primary cells needs to be studied further. As the structural conformity of these zinc compounds might have the ability to bind with DNA and inhibit transcription, we have tested the DNA binding of several transcription factors in presence of zinc compounds *in vitro*.

Indeed, all the zinc compounds have the ability to interfere with DNA-protein binding, as shown by NF-κB, p53 and AP-1 DNA binding with their respective consensus promoter binding site. This indicates that the zinc compounds in the present investigation are able to inhibit transcription binding ability and thus might block the expression of key proteins which are involved in cell division. This suggests that these compounds are potentially novel tumour therapeutic drugs. Further, toxicity tests on PBMC indicate no significant deleterious effect, confirming that the investigated zinc compounds may have therapeutic applications.

Conclusions

The syntheses of nine new neutral small molecules of zinc with (*E*)-*N*-(pyridin-2-ylmethylene)arylamine were successfully accomplished. These compounds have been characterized by single crystal X-ray diffraction analysis and spectroscopic methods. The Zn(II) compounds displayed distorted tetrahedral (**2**-**4**, **6** and **7**), square pyramidal (**8**) or trigonal bipyramidal (**9**) coordination geometries; in the last case, a dinuclear Zn(II) complex was obtained. Five water soluble dichlorozinc compounds (**1**, **3**-**5** and **8)** have been selected on the basis of a rational structure-based design approach which may have the ability to target and modulate tumour activities. The investigated zinc compounds exhibited *in vitro* tumour-inhibiting activities against the HeLa cell line that are much lower than that of cisplatin. However, cisplatin has enormous side-effects as an anti-cancer drug, while the higher concentrations of zinc compounds did not show any cytolysis, suggesting that they may exert less side-effects. Nevertheless, the bioavailability and degradation of these compounds in the biological system need to be determined before their use as therapeutics. The investigated Zn(II) compounds showed the ability to bind proteins, which is the requisite for a drug to act as an anticancer agent. The zinc complexes also exhibited significant cytotoxic activity toward the HeLa cell line and their high IC_{50} values did not induce any cytolysis effect. Binding studies indicated that the cell death induced by compound **4** may be caused by an apoptotic pathway through inhibition of binding of key transcription factors like NF-κB and AP-1 which are responsible for cell proliferation. Although the zinc compounds potently inhibited the p53 DNA binding, the tumour suppression may not be that important in the tumour biology since a lot of tumour cells have the non-functional p53. The studied zinc compounds predominantly inhibited the cell proliferative effect that occurs via inhibition of rate-limiting transcription factors, like

NF-κB and AP-1. Further studies are needed to understand the mechanism of action and the structure – cellular activity of this class of water soluble zinc compounds.

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